Privacy interest information

Subject:	Synergy
Location:	PY12621 -- Ph: [redacted] / Code: [redacted]
Start:	Thu 11/17/2016 3:00 PM
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Recurrence:	(none)
Meeting Status:	Accepted
Organizer:	Housenger, Jack
Required Attendees:	Keigwin, Richard; Kenny, Daniel; Laws, Meredith; Goodis, Michael; Cowles, James; Anita Pease; Nesci, Kimberly; Odenkirchen, Edward; Lowit, Anna
Optional Attendees:	Guilaran, Yu-Ting; Marietta Echeverria

11/15/16 Update: Conference Line

Ph: [redacted]
Code: [redacted]

From Dan Campbell of Syngenta: Attached is a draft paper written by Dr. Jason Belden of Oklahoma State University that will serve as the basis for our discussion Thursday. Dr. Belden is pulling together a slide set to facilitate the discussion of his proposed approach and the case studies from Syngenta data. We are looking forward to a great discussion on this interesting subject!

From Syngenta, the meeting will be attended by Dr. Belden, Dr. Richard Brain of Syngenta and myself.

Email invite from Syngenta:

Syngenta would like to meet with EPA to discuss the topic of synergy. We have been working with one of the preeminent research scientists in this area, Dr. Jason Belden of Oklahoma State University. He has developed an approach to assessing the potential for synergy, and has used Syngenta GLP data on a few case studies. We would like to meet with the folks at EPA (RD, EFED, PRD, others?) who are involved in evaluating this topic to discuss and get feedback on the approach and the case studies.

Thank you for your consideration of this meeting, and please let John and me know if there is anything we need to do to help arrange the discussion.
Kind regards,

Dan

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Incorporating the Joint Toxicity of Co-applied Pesticides into the Ecological Risk Assessment Process

Draft *Report to Syngenta Crop Protection – November 15, 2016*

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Introduction

Pesticides are frequently formulated as mixtures of active ingredients in order to allow for broader spectrum of pest control and reduction in pesticide resistance. However, when conducting ecological risk assessment (ERA), assessments are conducted primarily on individual active ingredients and potential joint toxicity of the mixture is generally assessed qualitatively. However, a great deal of research has been conducted on the toxicity of mixtures over the last few decades allowing for opportunities to include this as part of the ERA process.

As part of this research, numerous studies have investigated methods for predictively modelling the toxicity of mixtures based on the toxicity of individual toxicants. The conceptual foundation was developed early in the 20th century with original concepts described by Loewe and Muischnek (1926) and Bliss (1939). A complete framework was developed by 1969 (Hewlett). In the last two decades, numerous studies have extensively tested the predictability of these models for many species and contaminants. In addition, meta-analyses evaluating the accuracy of the modelling attempts have been conducted (Belden et al. 2007, Cedergreen 2014). It is clear from this research that mixtures of pesticides applied at equipotent concentrations typically result in more toxicity than would be expected from either of the individual pesticides by itself. It is also clear that predictive models are relatively good at estimating toxicity and cases of synergy, defined here as more than additive toxicity, are very infrequent (Belden et. al. 2007, Cedergeen 2014). Thus, there is a workable approach for incorporating joint action of co-applied pesticides into the risk assessment process based on existing models and research.

Recently, discussions have centered around patent applications that claim unexpected results based on better control of a pest with mixtures as compared to individual active ingredients. The approach used to make these patent claims is different than the approach more typically used to evaluate mixtures in ecotoxicology. Understanding the difference between disciplines provides context

between a patent claim and the ecological risk assessment. Moreover, in order to proceed with an ERA process that considers formulation mixtures, a framework that utilizes our current knowledge of mixture toxicity can be used to assess to assess potential implications to the risk assessment.

Thus, our objectives include: 1) describe differences between patent claim and ecotoxicological approaches for evaluating joint action of pesticides; 2) describe an approach for conducting risk assessment of jointly applied pesticides; 3) determine the deviation from a mixture model that would suggest greater than expected toxicity based on the impact of interlaboratory study variability during toxicity testing; and 4) demonstrate our approach using case studies for the appropriate risk assessment process for 31 formulation/species combinations.

Background - Models used for predicting and understanding the toxicity of mixtures

Approaches for predicting joint action are well defined and although specific mathematical assessment have improved, the general framework was outlined decades ago (Bliss 1939, Hewlett 1969). Within this framework, toxicants can act jointly or independently based on similar or dissimilar modes of action. In addition, toxicants could interact, which typically suggests one toxicant interfering with the biotransformation of the other. On the basis of this framework, we can describe mixture toxicity in practical terms within three categories: Concentration Addition (CA), Independent Action (IA), or Simple Interaction (SI) (Belden et al. 2007).

Potentially the most useful and widely used category is CA originally proposed by Loewe and Muischnek (1926). CA is based on the assumption that all chemicals in a mixture have the same mode of action. CA is thought to occur when the toxicants can act as dilutions of each other, meaning that the effect of one toxicant can be replaced totally or in part by the equally effective amount of another toxicant (Altenburger et al. 2000). CA is frequently described by the following model:

$$ECx_{mix} = \left(\sum_{i=1}^{n} \frac{p_i}{ECx_i} \right)^{-1} \quad (1)$$

where ECx_{mix} is the total concentration of the mixture that causes x effect; p_i indicates the proportion of component i in the mixture; and ECx_i indicates the concentration of component i that would cause x effect.

In practice, CA is frequently described using a normalized toxicant concentration scale (Toxic Units, TU) that allows addition of the concentrations of each toxicant. TUs can be calculated as follows:

$$\text{sum TU} = \sum_{i=1}^{n} \frac{C_i}{ECx_i} \quad (2)$$

where, C_i is the exposure concentration of the *i*th chemical in the mixture, EC_{xi} and the other variables are defined as stated for Equation 1. Thus, a TU is mathematically identical to a risk quotient (RQ) and a summation of risk quotients is equal to sum TU.

The second category is based on IA following the concept that toxicants that have dissimilar mode of action will act completely independently. Conceptually, this model is a statistical approach to predict the likelihood that one of multiple possible events will occur. Accordingly, the effect of the total mixture concentration can be predicted by the expected effect of each component, using the following equation (Berenbaum 1985):

$$E(c_{mix}) = 1 - \prod_{i=1}^{n} (1 - E(c_i)) \quad (3)$$

where $E(c_{mix})$ is the total effect of the mixture and $E(c_i)$ is the effect expected from component *i*. IA can alternatively be expressed for a binary mixture as follows (Colby 1966):

$$E = (A + B) - \frac{A \times B}{100} \quad (4)$$

where, A is the % pesticide control of pesticide A, B is the % pesticide control of pesticide B, and E is the % expected pesticide control. This form is typically used as a screen for efficacy measurements and in the patent process.

The third category (SI) is based on the classical concept of a "synergist". An example of SI is piperonyl butoxide (PBO) and pyrethroid insecticides. Although PBO is applied at concentration where its individual toxicity is not important, it inhibits detoxifying enzymes (cytochrome p450s) causing the pyrethroid to have a greater effect and thus resulting in a joint effect greater than would be expected. Interactions cannot be currently predicted and are not expected to occur frequently. Although this has been a useful category for experiments focused on synergy, since it is not predictive there is little value towards development of a predictive framework. Thus, no specific model is used in this proposed approach other than CA and IA.

Experimental results will vary from predicted results based on CA and IA for many reasons including interactions, variability between individual and mixture toxicity tests that were not conducted concurrently, and lack of mechanistic fit of the model. A simple approach to compare between empirical results of mixture toxicity and predicted methods is the Model Deviation Ratio (MDR) described by the following equation (Belden et al. 2006):

$$MDR = \frac{Expected}{Observed} \qquad (5)$$

where *Expected* (ECx_{MIX}) is the effective concentration of the mixture that would be predicted by the model and *Observed* (ECx_{OBS}) is the effective concentration for the mixture obtained from toxicity testing.

Out of these conceptual categories, CA has been most widely used and promoted for use as a general utility model for several reasons. First, CA is conceptually simple and is parallel with risk

assessment approaches. As noted, TU is mathematically identical to RQ. Second, CA has been found to be strongly predictive. Ninety-five percent of experiments testing the predictability of CA found that MDR values were less than 2.58 and only 1% of experiments were greater than 4.19 (n=207, Belden et al. 2007). Typically, CA is slightly more environmentally conservative than IA especially with mixtures that have different modes of action and for steeper slopes (Drescher and Boedeker, 1995)

How model selection can influence the perception of synergy

The suggestion that a mixture is synergistic has to be premised on the idea that there is a predicted baseline of toxicity for the mixture (Cedergreen 2014). In the case of patent registration, baseline assumes IA using the Colby Equation (Equation 4). In contrast, most ecotoxicological efforts have promoted CA (Belden et al. 2007, Cedergreen 2014, EFSA 2013). In order to demonstrate how method selection can impact the results, we provide a theoretical example. Mixture AB is composed of two toxicants (A, B) and the relative concentration of the two components was mixed at a ratio of the respective EC50 values resulting in 1:2 (A:B) to obtain equipotency. Both compounds have a relatively steep concentration-response curve (Figure 1). Assuming concentration addition and using Equation 1, we can obtain a pointwise estimate for the toxicity of the mixture (AB, Figure 1). As would be expected the relative toxicity of the mixture is intermediate to the components. For the mixture AB at concentration of 2.1 (0.7 A and 1.4 B) we would expect 75% effect (Figure 1). If we apply the typical formula used in patent work (Colby Equation, Equation 4), we would expect that 0.7 of A would result in 27% effect and 1.4 of B would result in 32% effect. The joint effect would be predicted to be 50%. In this case, a mixture that would be characterized as additive and well described by the CA model would be characterized as synergistic based on the Colby equation.

All mixtures where the concentration response curves of the components have a relatively steep slope would result in findings similar to our theoretical example and for shallower slopes the models tend to converge (Drescher and Boedeker, 1995, Backhaus et al. 2004). Comparison between these models has been well characterized in previous work and the factors that determine the quantitative difference between the models includes the number of components, the ratio of the components, and the slope of the concentration–response curves (Drescher and Boedeker, 1995). A larger number of components will enhance the differences between models as has been demonstrated for 14 dissimilar acting pesticides where the experimental data matches IA, yet CA over predicts toxicity 3x (Backhaus et al. 2000). Similarly, the toxicity of a set of 16 similarly acting toxicants, all uncouplers of oxidative phosphorylation, were well predicted by CA and under predicted by IA by a factor of 3x.

In many cases CA has been found to be generally predictive of toxicity even with compounds that have different modes of action (Belden et al 2007). This is potentially due to more generalized physiological effects such as ion imbalance in fish or oxidative damage in plants being the proximal mode of action (Aslop and Wood 2013). It is likely that the toxicity of many co-applied pesticides within a formulation will be predicted to be lower than observed based on IA and the Colby Equation, yet well predicted by CA. Thus, despite the label of synergy, the ecotoxicity of many formulations will be adequately predicted based on an approach using CA. CA has been commonly suggested for use when conducting ERA with mixtures, especially for first tier assessments (Backhause and Faust 2012; Chevre et al. 2006; EFSA 2013) and will be the primary model in our framework.

Theoretical framework for conducting an ERA of co-applied pesticides

Based on knowledge of the risk assessment of the individual active ingredients and predictability of mixture models, the amount of empirical testing that is merited can be reduced. If all active

ingredients have very low risk, it is very unlikely that their mixture would result in increased toxicity to a degree that would impact the risk assessment. Similarly, if testing is merited, it should be targeted at test organisms that are the most sensitive to the active ingredients as this is the scenario where the presence of a mixture could potentially change the evaluation of risk when compared to the components of the mixture. Our proposed framework incorporates these considerations and is provided by a flow chart (Figure 2) and rationalized below.

Threshold for requiring empirical testing

The first step is to decide whether the active ingredients in the mixture are close enough to the estimated environmental concentration (EEC) that any detailed consideration of mixtures is warranted. If the predicted toxicity of the mixture, expressed as a lethal or effective concentration, is sufficiently above the EEC, then there is very little risk posed by the mixture. This relationship between ECx_{mix} and the EEC can be expressed as sum TU (Equation 2), which is equivalent to a sum RQ and will be referred to as Sum RQ. Previous work has suggested that synergism exceeding an MDR of 5 is uncommon. The 99 percentile MDR value reported in Belden et al. (2007) was 4.19. Moreover, in a review of synergism, Cedergreen (2014) only identified a total of 19 studies that exceeded an MDR of 5 from well over 200 studies conducted. Thus, we have set an uncertainty factor of 5x for potential synergy. If the Sum RQ is greater than 1/5 the Level of Concern (LOC), then empirical testing would be warranted and below 1/5 the LOC then it would not be warranted (Supplemental Information Table S2). The emphasis for empirical follow up testing should be on environmental receptors that are the most sensitive (highest Sum RQ values in relationship to LOC).

Due to the greater potential of synergism by some pesticide classes, an exception to the Sum TU cutoff should be considered. If one of the pesticides is in a class of compounds and at a concentration that is frequently associated with greater toxicity than predicted by the CA models, then empirical testing would be recommended. Azole fungicides and cholinesterase inhibiting insecticides accounted

for 95% of empirically known synergistic mixtures (Cedergreen 2014). All 19 of the studies identified by Cedergreen (2014), which exceeded an MDR of 5, include a pesticide from one of these classes. Thus, all mixtures as reviewed by Belden et al (2007) and Cedergreen (2014) that exceeded an MDR of 5 would be empirically tested based on this recommendation.

For cases where empirical testing is not merited, a final step is to consider whether only one component accounts for nearly the entire sum RQ (all others are less than 5% of the sum RQ). If this is the case, then the formulation mixture ERA could defaults to the ERA of the most toxic component.

Comparison of empirical testing to CA model

Following empirical testing, a comparison between the experimentally determined effective concentration (ECx_{OBS}) is made to the CA modelled value (ECx_{MIX}) and an MDR is calculated. An MDR of exactly 1 is not expected to occur frequently given the potential for variability during testing. There are several potential approaches to determine the magnitude of an MDR that would suggest synergism. Previous work has used an MDR of 2 (Belden et al 2007, Cedergreen 2014) or 5 (EFSA 2013). However, these values have limited conceptual underpinnings. It is also possible to use a value based on the results of meta-analysis of mixture data. For example, the 95th percentile occurrence of MDRs in Belden et al. (2007) was 2.58 and thus MDR values outside of this range would be well beyond the normal result. However, this may be a skewed approach as these data sets include synergistic mixtures and are not representative of the potential variability that would be obtained for additive mixtures only. It is possible that more than 5% of mixtures are synergistic biasing the acceptable MDR. The third approach, which we selected for this investigation, is to set the acceptable magnitude of deviation based on how likely it is that the MDR value is higher than could be explained based on experimental variability alone.

Testing of formulations containing co-applied pesticides will frequently occur at different times and potentially in a different laboratory than the individual active ingredients. Determining how much impact intra- and inter-laboratory variability may have on MDR calculations is necessary to determine

how likely it is that the MDR suggest synergy rather than variability among studies. In order to calculate MDR values that would commonly occur based on likely variability, we conducted the following exercise. First round robin or planned inter-laboratory comparison studies were identified from the literature using species and endpoints as similar as possible to what would be used for ERA of pesticides. Twelve studies were identified with coefficient of variation (CV) ranging from 1.8-180 (Table 1). Additionally, searches of the EPA ECOTOX Knowledgebase (website) were made for commonly used pesticides and endpoints and CVs were calculated ranging from 44-221 (Table 1). Toxicity tests conducted for pesticide ERA are likely more controlled and less variable than comparing studies in the ECOTOX database. However, we would suggest that they are similarly or less controlled across labs and time than would be expected from a preplanned round robin study. Based on this survey of data, we propose that a CV of 100% would be likely representative of the intra- and inter-laboratory variability. This is at the higher end of CV values found in round robin studies and at the bottom end of data collected from the EPA ECOTOX Knowledgebase.

Next, distributions of likely MDR values based on CV values were determined through a modelling exercise. We assumed a log-normal distribution and randomly generated effective concentrations for theoretical active ingredients and a formulation mixture centered around a "true" value of 1 (12,000 iterations). For each iteration, an MDR was calculated using Equation 5. The distribution of the resulting MDR values were then determined. We repeated this process for CVs of 60, 100, and 140%. Full methodological details are provided in Supplemental Data. We used an extended range of CV values instead of only 100% to provide insight into how much this assumption shifts the outcome. We also conducted the analysis for single, binary, and tertiary mixtures. Although binary are common, tertiary or greater mixtures will occur in formulations. Single compound modelling provides insight into the scenario where a single active ingredient is driving toxicity. As would be expected, the

distribution of MDR values spreads to greater extremes as the CV value increases (Figure 3). MDR values were the highest for binary mixtures (Table 2).

Based on a CV of 100% and 95% level of likelihood (only 5% of samples would randomly exceed the threshold) a MDR of 4.70, rounded to 5, was identified for use as a threshold (Table 2). MDR values that exceed this value would be considered synergistic and below would be considered additive. If the MDR exceeds 5, then it is recommended that ERA steps include using the mixture results obtained through empirical testing (ECx_{OBS}). If the MDR value is below 5, then the mixture is assumed to fit the CA model and ERA continues based on the assumption of additive toxicity. If the MDR is below 5 and only one component accounts for nearly all TUs (all others less than 0.05 TU) then the formulation mixture ERA could default to the ERA of the most toxic component as there would limited difference in the assessment.

Case studies using pesticide mixtures

In order to evaluate the approach, 31 formulation mixtures that have been previously tested for toxicity were identified as well as the complementary individual toxicity studies (Table 3 and S3). Studies were identified based on availability of data for individual and mixture toxicity results. These data were collected prior to development of this framework and many of these studies would not have triggered empirical toxicity testing based on the 1/5LOC threshold. Each study was conducted under good laboratory practices using standardized guidelines. For each study, expected toxicity of the formulation mixture was predicted based on CA, compared to the empirical toxicity test result, and an MDR was calculated. MDR values ranged from 0.16-9.69 (Table 3) and were relatively symmetric between less-than and greater-than predicted by the CA model (Figure 4). This even distribution may suggest that much of the deviation from the model is due to testing variability rather than interactions. It would be less likely that frequent interactions would result in a uniform distribution, especially since

lower MDR values were typically noted in studies where toxicity tests were typically conducted within a single method design instead of across laboratories (Belden et al. 2007).

Ideally, all studies for individuals and mixtures would have definitive effective concentrations in order to most accurately calculate MDR values. However, in 19 of 31 studies at least one test reported a greater than value as concentrations high enough to cause an effect were not tested. For 7 studies, flagged F, the formulation did not have a definitive effective value. For these studies the MDR could be biased high. For example, if the nondefinitive testing was stopped at 54 g/ha (see mixture study 28 in Table S2) but require 4x this value (216 ng/ha) to obtain a definitive result, then the MDR value would be biased 4x to high. These data are not very useful for this assessment as the bias is certain and we have excluded them from further analysis. Regardless, the likely reason that higher concentrations were not performed is that the concentrations were at the limit typically tested (e.g. 2000 mg/kg for avian acute) and thus these are typically cases where toxicity would be limited. This scenario will not be common if this framework would be adopted as empirical mixture testing would not typically need to be performed. For other studies (n=12), one of the individual toxicants had a greater than value for the effective concentration. In these cases, the bias is not as significant as the deficient test was typically for an active ingredient that was of too low a potency to greatly influence the predictive toxicity of the mixture. In only 3 cases, the TU values associated with the non-definitive tests were over 0.10 and even in these cases the bias is not expected to exceed 50%. Thus, these data were considered valid despite potential for an MDR to be biased low.

Although many of these formulation mixtures would not have exceed the 1/5LOC threshold and testing would not have been needed, we assumed for this illustration that all 24 studies require empirical testing to allow estimation of the frequency that a formulation mixture will exceed the MDR threshold of 5. Of the 24 samples, only 3 exceed the threshold and recommendations would be that the risk assessment based on the results of the formulation mixture toxicity test. Of the 21 studies that did

not exceed the threshold, 11 had only a single active ingredient that accounted for more than 5% of the RQ (Table 3 flagged with S). Thus, these 11 could default to the ERA based on the single most toxic active ingredient. Risk is driven almost completely by a single pesticide and there will not be a significant difference between RQ for this single pesticide and the sum RQ. For the other 10, the ERA would assume additivity and thus be based on CA modelling of toxicity derived from individual active ingredients (ECx_{MIX}).

As a case study, the toxicity of a mesotrione and s-metolachlor formulation was evaluated for risk to nontarget plants. A detailed example is provided in Table 4 to demonstrate the process. As a first step of the process the RQ for both individual pesticides was determined along with a sum RQ. EEC values were estimated using TerrPlant 1.2.2 to provide a simple example for plants, system specific and higher tier models would be used as appropriate. In this case, the sum RQ value well exceeds the threshold value (1/5 the LOC, which in this case the LOC is 1.0 and thus the threshold is 0.2). Therefore, empirical testing would be recommended. Based on empirical testing the MDR value was 5.3, which exceeds the suggested cutoff of 5.0, which is suggestive of synergy. Therefore, it would be recommended that risk assessment utilizes the empirically determined toxicity for the formulative mixture. If the expected ratio of the EEC differs from that used for the mixture toxicity test, toxicity can be calculated for the new ratio using CA (Equation 1) and the toxicity adjusted by the MDR.

This process would simultaneously be conducted for all toxicity testing species of interest within a risk assessment. For example, this same mesotrione and s-metolachlor formulation would be evaluated for a series of plant species (Table 5). For the seven species we use as examples, there is a range of sum RQ values. Five of seven values exceed the 1/5LOC threshold and thus empirical testing will be required. However, three species (cucumber, lettuce, and tomato) have much higher RQ values than onion and soybean (greater than 5x). Thus, empirical testing would be recommended for cucumber, lettuce, and tomato as it is highly unlikely that the testing of onion and soybean would result

in enough synergism to impact the ERA as compared to the most sensitive species. Following empirical testing of the three species, each species is considered individually throughout he framework. For cucumber, the MDR was less than 5 and thus additivity is assumed, despite potential antagonism. Based on CA, it is appropriate to use sum RQ for the risk assessment. However, since mesotrione accounts for near 99.3% of the RQ, then it would also be appropriate for this species for the risk assessment to be based on mesotrione only as there would not be a difference (6.28). Lettuce follows this same pattern and the RQ for mesotrione can be used for risk assessment (16.4). However, as previously described the RQ for tomato is based on empirical testing and due to the potential synergy the RQ is elevated from 11.7 to 63.1. The result is tomato being the most sensitive species and consideration of the mixture increasing the estimated risk.

Discussion of the framework

In order to create the framework there were several assumptions and choices that were made that could influence outcomes. Our goal was to balance risk assessment conservatism with reducing testing that would likely not result in improvement of the ERA process.

Establishing MDR limit based on variability

As shown in Table 2, the difference in potential MDR values between CVs is not excessively large. However, selection of a different CV would change the MDR threshold. It would be enlightening to have better round robin data among laboratories that commonly conduct pesticide toxicity tests to see how much variability exists. Moreover, if some representative formulation mixtures were able to be determined within the same test design as individual active ingredients, then our ability to determine additivity versus synergistic results could be evaluated. The frequency of studies exceeding a MDR of 2 in the case studies examined was 21% (5 of 24; Figure 4), which is a little higher than reported in meta-analysis (7%, Cedergreen 2014). The majority of mixture studies in the literature tend to be uniform

mixture designs that allow statistical comparisons. These studies will frequently have less variability and greater ability to statistically resolve differences between empirical data and the models.

Using modelling data for mixture ERA when empirical data exists

If the toxicity of formulation mixtures was found to be additive (MDR<5), we suggest to proceed with the ERA assuming additivity and using CA. It could be argued to either use this modeled value or the empirically measured toxicity value (ECx_{OBS}). Variability in testing could result in either higher or lower than predicted toxicity and there will not be certainty whether the individual toxicity tests or the mixture test is more accurate. Since additivity was found, the values will not be meaningfully different. However, it is important to choose *a priori* which route should be taken to ensure greatest certainty, depending upon the case being assessed.

Assessing environmental mixtures beyond formulations

Our approach as written only considers mixtures formulated together. It is possible that more complicated mixtures can occur for short periods of time in certain environments due to tank mixes or multiple products being applied in a watershed. Consideration of formulation mixtures is a necessary first step and potentially the most important component in considering pesticide mixtures. If specific tank mixtures are likely, such as the case where potential mixtures are listed on the pesticide label, then the framework utilized here can be directly used to also evaluate those mixtures. Although not all tank mixtures can readily be identified as pest control approaches used at the field level are not always predictable, common tank mixtures are likely identifiable and can be assessed. As formulation mixtures are tested more frequently, more will be known about possible interactions among classes of pesticides and weight of evidences approach can be developed that will help identify tank and environmental mixtures that are of the greatest likelihood to be of greater environmental concern.

Broader environmental mixtures that may occur for short periods of time in streams and rivers receiving runoff from many fields with potentially different crops and pest control systems may be

harder to incorporate into any framework, but are also less likely to be of high concern. Although environmental mixtures occasionally can be composed of multiple contaminants most of these compounds are at much lower TUs as compared to the most potent component(s) that are likely the drivers of toxicity (Belden et al 2007B, Trimble et al. 2009). Potential environmental mixtures where toxic components vary greatly from site to site and each active ingredient is registered to different agrochemical companies will profoundly increase complexities of the assessment (Lydy et al. 2004). The resources spent towards this level of ERA may not be warranted based on the potential environmental risk.

Classes of pesticides that need closer scrutiny

Both azole fungicides and organophosphate insecticides (OPs) can potentially change the toxicokinetics of other compounds through inhibition of cytochrome p450 enzymes (Azole fungicides; Cedergreen et al. 2006) or esterases (organophosphate insecticides; Belden et al. 2006) in some organisms. In addition, many OPs are proinsecticides; thus, toxicity is dependent on toxicokinetic activation potentially resulting in more sensitivity to changes in toxicokinetics. For example, triazine herbicides tend to increase the toxicity of some OPs (Belden et al. 2000) despite not occurring commonly in synergistic mixtures (Cedergreen 2014). Thus, these classes were identified in the framework to trigger empirical testing due known modes of action with the greater chances of synergism occurring. However, consideration of these groups should also account for potential exposure concentration. For instance, OP insecticides tend to be synergistic at higher concentrations when grouped with pyrethroids, but not at lower concentrations (Belden et al 2006). Many studies investigating azole fungicides were conducted using equipotent method designs resulting in higher than environmentally relevant concentrations of the compounds being tested (Bjergager et al. 2016). If the EEC for these groups is below concentrations required to cause enzyme effects and synergy, empirical testing may not be needed. Additional testing of representative pesticides from each class may be

needed to find concentration thresholds that could result in synergy. For example, recent work was conducted that identified the threshold concentration of several azole herbicides needed to synergize pyrethroid insecricides in *Daphnia magna* and found that typically the concentrations were above what would be typically found in the environment (Bjergager et al. 2016).

Effect of Pesticide Ratios and Testing Concentrations

Most environmental exposure of pesticides will occur in ratios that are not equipotent. From the current case studies, 17 of 31 mixtures had a single active ingredient that accounts for the majority (>95%) of the sum RQ. Interestingly, formulative mixtures in these scenarios may result in lower RQ than a formulation containing the more potent pesticide by itself. Typically, the application rate is lower for an individual active ingredient in a formulation containing a mixture, thus the exposure level of the more potent component will be lower. Given that the RQ is driven by this single component, risk will be less if there is no synergy.

The concentration that is required to cause an effect may influence the likelihood that there is synergy. As required concentration become higher, the potential to overload detoxifying systems or inhibit detoxifying enzymes through secondary modes of action will increase. Thus, it is likely that synergy will occur more readily for species that are less sensitive. However, the present of synergy for these species is not likely to change the overall risk assessment as deviation from the additivity will likely still be below an MDR of 5.

Comparisons to other mixture ERA efforts

Our proposed framework has many similarities to the approach described by the European Food Safety Authority (EFSA 2013). Both approaches use CA as the primary model and a classification system built around MDRs. The primary conceptual difference between approaches is the initial threshold step to determine if empirical testing is needed. Resources should not be utilized in scenarios where there is minimal predicted risk based on individual active ingredients and there is no precedent for the

magnitude of synergy that would be required to change the outcome of the risk assessment. Our MDR threshold for CA to be considered predictive was set at 5 based on assessment of variability among tests. This threshold is exactly the same as established by EFSA (2013).

Several studies have suggested approaches incorporating a combination of CA and IA models (Backhaus et al. 2012). Theoretically this type of approach is more complex than the simplified approach we propose. However, a lack of knowledge in regard to the mode of action of many pesticides to nontarget organisms, such as herbicides toxicity to daphnia, and the overall high predictability of CA despite different modes of action makes CA only the best approach.

Endpoints and exposure scenarios to be tested

There are often specific endpoint/exposure/ test species scenario combinations that clearly drive the risk for most formulations. As noted, mixture toxicity should be targeted at those scenarios. In addition, if Tier 1 mixture testing suggests no synergy, an argument can be made that it is unlikely that higher-tier testing with the same organism would result in synergy. Since most known mode of action synergy is due to toxicokinetics changes triggered by one of the toxicants, it would be expected this would also occur in acute tests where concentrations are higher. Future research is needed to support this hypothesis.

Conclusions

Co-applied pesticides are important to evaluate as mixtures due to the potential co-occurrence in the environment. Due to the predictability of mixture models, empirical testing of mixtures may not need to be conducted if the expected effective concentration of the mixture is much higher than the estimated environmental concentration of the formulation. Where empirical testing might be merited, if measured toxicity is within a factor of 5, then it is likely due to a combination of additivity and/or testing

variability and synergy is not concluded. Further, if the toxicity of a mixture is driven by a single component and no synergy is noted, the risk assessment of the single compound is likely sufficient. Otherwise, the empirically measured toxicity of a synergistic mixture and the CA modelled toxicity of an additive mixture should be used as the effective concentration during further risk assessment. In all, the described approach appears to be useful for risk assessment of formulation mixtures based on the assessment of 31 case studies.

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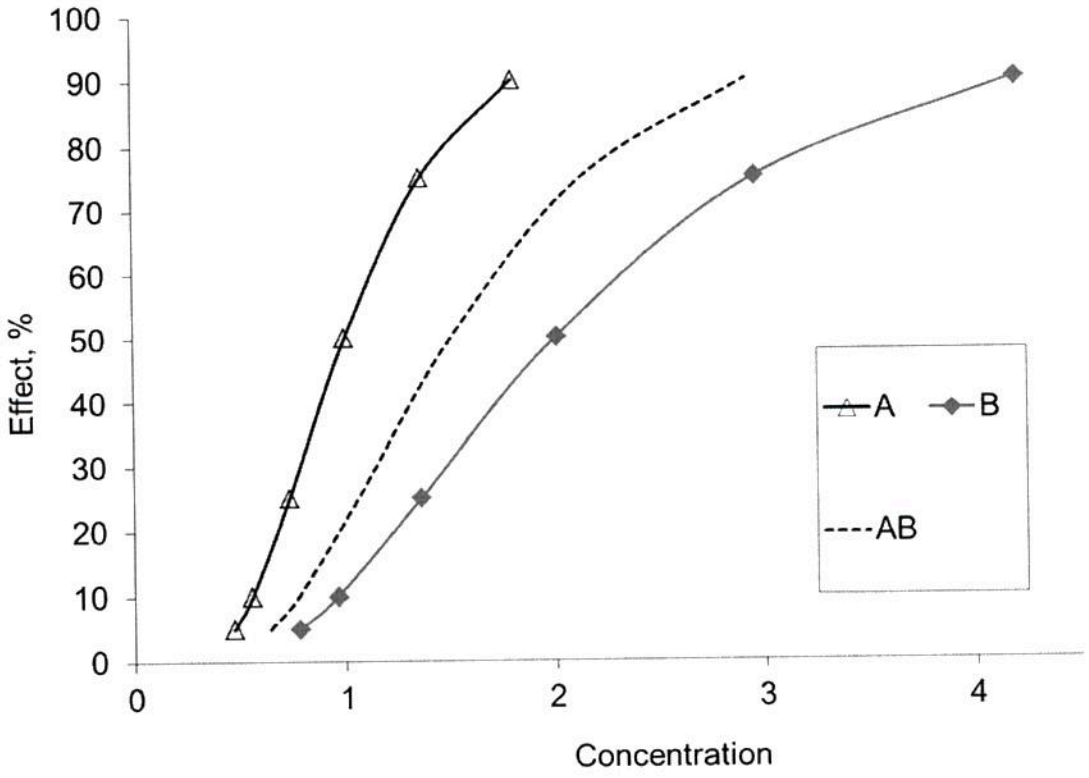

Figure 1. Theoretical concentration-response curves for two individual components A and B, and a 1:2 mixture of A and B (AB).

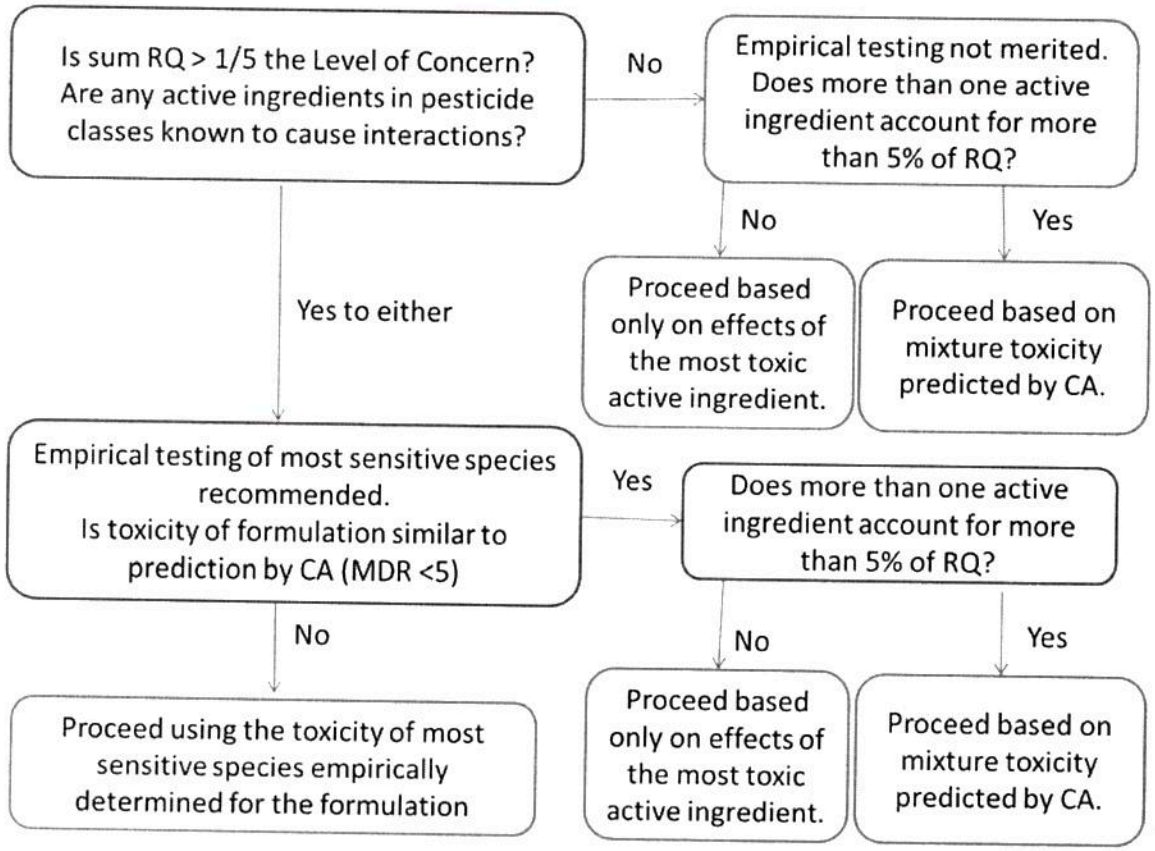

Figure 2. Flow chart illustrating the proposed theoretical framework for conducting an ERA of co-applied pesticides.

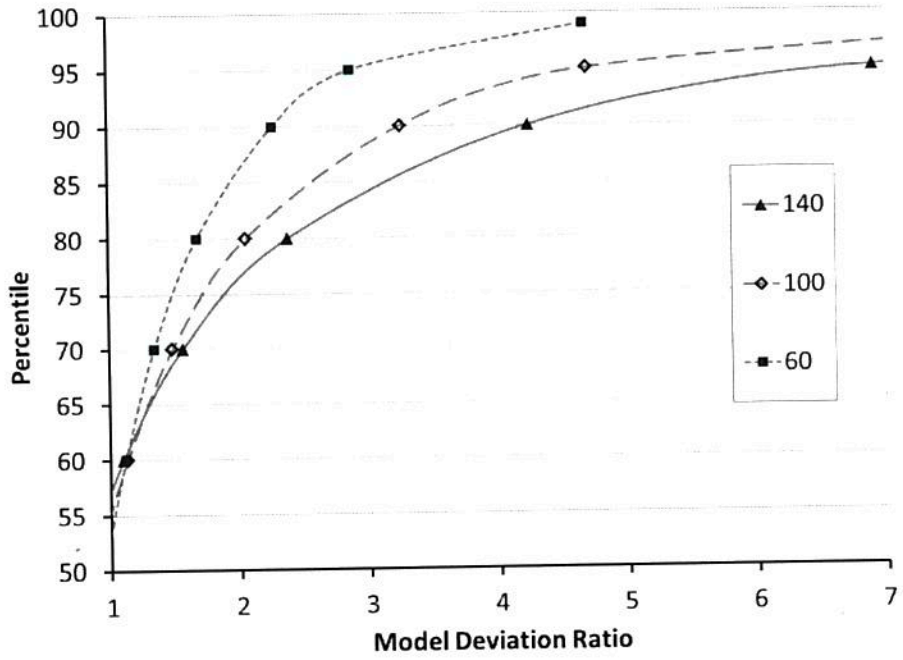

Figure 3. Cumulative distribution of model deviation ratios that would occur based on variability among tests. Results shown are for a binary mixture and 60, 100, and 140% coefficients of variation.

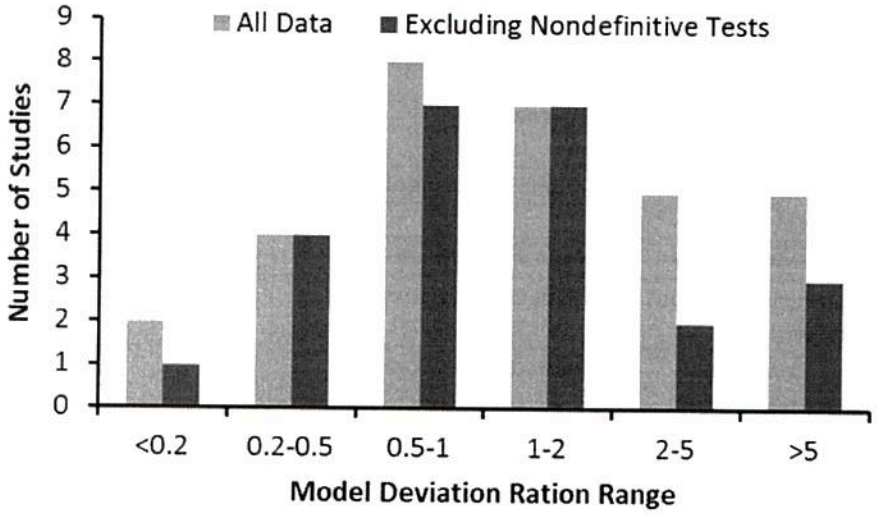

Figure 4. Number of formulation mixtures studied that had a model deviation ratio (MDR) within the provided range. Excluding Nondefinitive Tests describes the same data set as All Data (n=31), except studies with nondefinitive effective concentrations of the mixture were excluded (n=24).

Table 1. Examples of interlaboratory variability measurement. The first 12 examples are from round robin or otherwise planned studies, typically using a standard toxicant. The last three studies are sets of data obtained from the ECOTOX Knowledgebase.

Reference	Organism	Toxicant	Number of Labs/Reports	CV
Weltje 2011	*Chironamus* – Survival	3,5 dichlorophenol and KCL	13-15	36-60
Ronco et al. 2002	*Daphnia* lethality	Mean of three toxicants	8	32
Ronco et al. 2002	*Lactuca* - Seed root inhibition	Mean of three toxicants	8	64
Ronco et al. 2002	*Selenastrum* cell growth inhibition	Mean of three toxicants	8	59
Busquet et al. 2014	Zebrafish acute	21 Tested	3-7	1.8-56
DeGraeve et al. 1991	*Pimelphales* survival	Sodium pentachlorinate and potassium dichromate	10	24-44
DeGraeve et al. 1991	*Pimelphales growth*	Sodium pentachlorinate and potassium dichromate	10	28-88
Burton et al. 1996	*Hyalella* – survival	KCL	10	23
Burton et al. 1996	*Chironomus* – survival	KCL	10	53
Norberg-King et al. 2006	*Hyalella* – survival	Contaminated sediment	11-14	5-170
Norberg-King et al. 2006	*Chironomus* – survival	Contaminated sediment	7-15	5.6-33
Norberg-King et al. 2006	*Chironomus* – growth	Contaminated sediment	6-12	32-60
ECOTOX Knowledgebase	*Daphnia* lethality and immobility, 48h LC50 or EC50	Atrazine	14	221
ECOTOX Knowledgebase	*Daphnia* lethality and immobility, 48h, LC50 or EC50	Lambda cyhalothrin	6	95
ECOTOX Knowledgebase	*Daphnia* lethality and immobility, 48h, LC50 or EC50	Metolachlor	7	44
ECOTOX Knowledgebase	*Lactuca sativa*, 28d biomass EC25	Atrazine	5	141
ECOTOX Knowledgebase	*Lactuca sativa*, 21d biomass EC25	Metolachlor	3	150

Table 2. Based on interlaboratory variability expressed as coefficient of variation (CV), distributions of likely MDR values for additive mixtures that would be obtained based on experimental variability alone were determined. 95th percentiles of those distributions are shown. The bold value emphasizes the threshold selected for use in this framework.

Intertest Variability, CV	60	100	140
Single Component	2.13	2.78	3.23
Binary Mixture	2.86	**4.70**	6.90
Tertiary Mixture	2.66	4.03	5.43

Table 3. Toxicity tests performed on 31 formulations that contained more than one active ingredient and the deviation of the empirically tested result to that expected based on concentration addition model. MDR – model deviation ration. T flag indicates that one of the active ingredients did not cause effect at the highest concentration tested. F flag indicates that the formulation did not cause effect at the highest concentration tested and thus the MDR is biased high. S flag indicates that only one of the active ingredients accounted for more than 5% of the sum risk quotient.

Species	Toxicant 1	Toxicant 2	Toxicant 3	Endpoint	MDR	Flag
Daphnia magna (Water flea)	Lambda-cyhalothrin	Thiamethoxam		Mobility/lethality, 96h	5.39	T, S
Daphnia magna (Water flea)	Mesotrione	Atrazine		Mobility/lethality, 96h	0.16	F, S
Daphnia magna (Water flea)	Bicyclopyrone	Metolachlor		Mobility/lethality, 96h	3.62	T
Daphnia magna (Water flea)	Cyantraniliprole	Thiamethoxam		Mobility/lethality, 96h	1.62	T, S
Daphnia magna (Water flea)	Mesotrione	Atrazine	Metolachlor	Mobility/lethality, 96h	0.53	
Pseudokirchneriella subcapitata (Green algae)	Mesotrione	Atrazine		Growth Inhibition, 96h	0.79	S
Pseudokirchneriella subcapitata (Green algae)	Bicyclopyrone	Metolachlor		Growth Inhibition, 96h	0.49	
Pseudokirchneriella subcapitata (Green algae)	Mesotrione	Atrazine	Metolachlor	Growth Inhibition, 96h	0.37	
Oncorhynchus mykiss (Trout)	Lambda-cyhalothrin	Thiamethoxam		Lethality, 96H	0.24	T, S
Oncorhynchus mykiss (Trout)	Bicyclopyrone	Metolachlor		Lethality, 96H	3.99	T, S
Oncorhynchus mykiss (Trout)	Cyantraniliprole	Thiamethoxam		Lethality, 96H	0.54	F, T
Oncorhynchus mykiss (Trout)	Mesotrione	Atrazine		Lethality, 96H	0.72	T, S
Oncorhynchus mykiss (Trout)	Mesotrione	Atrazine	Metolachlor	Lethality, 96H	1.49	T
Apis mellifera (Honey bee)	Lamba-cyhalothrin	Thiamethoxam		Oral Lethality, 48h	0.86	S
Apis mellifera (Honey bee)	Cyantraniliprole	Thiamethoxam		Oral Lethality, 48h	0.60	S
Colinus virginianus (Quail)	Cyantraniliprole	Thiamethoxam		Oral Lethality, 48h	2.17	F
Colinus virginianus (Quail)	Lamba-cyhalothrin	Thiamethoxam		Oral Lethality, 48h	3.39	F
Cucumus sativa (Cucumber)	Mesotrione	Atrazine	S-Metolachlor	Foliar Dry Mass, 21D	1.94	
Cucumus sativa (Cucumber)	Mesotrione	S-Metolachlor		Foliar Dry Mass, 21D	0.12	S
Lactuca sativa (Lettuce)	Mesotrione	Atrazine	S-Metolachlor	Foliar Dry Mass, 21D	0.71	S
Lactuca sativa (Lettuce)	Mesotrione	S-Metolachlor		Foliar Dry Mass, 21D	2.61	F, S
Avena sativa (Oat)	Mesotrione	Atrazine	S-Metolachlor	Foliar Dry Mass, 21D	1.99	T
Avena sativa (Oat)	Mesotrione	S-Metolachlor		Foliar Dry Mass, 21D	0.34	T
Allium cepa (Onion)	Mesotrione	Atrazine	S-Metolachlor	Foliar Dry Mass, 21D	9.69	F, S

Allium cepa (Onion)	Mesotrione	S-Metolachlor		Foliar Dry Mass, 21D	0.62	T, S
Lolium perenne (Perennial Ryegrass)	Mesotrione	Atrazine	S-Metolachlor	Foliar Dry Mass, 21D	6.32	T
Lolium perenne (Perennial Ryegrass)	Mesotrione	S-Metolachlor		Foliar Dry Mass, 21D	1.13	T
Glycine max (Soybean)	Mesotrione	Atrazine	S-Metolachlor	Foliar Dry Mass, 21D	5.09	F
Glycine max (Soybean)	Mesotrione	S-Metolachlor		Foliar Dry Mass, 21D	1.40	S
Lycopersicon esculentum (Tomato)	Mesotrione	Atrazine	S-Metolachlor	Foliar Dry Mass, 21D	1.80	S
Lycopersicon esculentum (Tomato)	Mesotrione	S-Metolachlor		Foliar Dry Mass, 21D	5.34	S

Table 4. Detailed example of using the mixture assessment framework to guide ERA process. Data shown for a formulation containing mesotrione and s-metolachlor with toxicity to *Lycopersicon esculentum* (Tomato). Each question is listed in a stepwise process using the framework provided in Figure 2. [1]Estimated environmental concentration based on ground application, no incorporation, and runoff to dry land using TerrPlant 1.2.2. [2]Predicted ER_{50} determined using concentration addition expressed in terms of formulation mass (Equation 1).

Question	Criteria	Required Data	Data Synthesis	Outcome
Does empirical testing need to occur?	Is Sum RQ > 1/5 LOC? (1/5 x 1.0 = 0.20)	ER_{50} Mesotrione = 1.3 S-Metolachlor = 2800 EEC[1] Mesotrione = 15.3 S-Metolachlor = 154	Individual RQ Mesotrione = 11.7 S-Metolachlor = 0.056 Sum RQ = 11.8	Requires Empirical Testing
Is toxicity of formulation similar to prediction based on CA?	Is MDR <5?	Predicted[2] ER_{50} = 34.2 Experimental ER_{50} = 6.4	MDR = 5.3	Not predicted by CA
Does more than one active ingredient account for more than 5% of Sum TU?	Only one active ingredient > 5% of Sum TU	Individual TU Mesotrione = 11.7 S-Metolachlor = 0.056 Sum TU = 11.8	Percent of Sum TU Mesotrione = 99.5 S-Metolachlor = 0.5	Single analyte driven.

Table 5. Example of data generated using the mixture assessment framework to guide ERA process for a list of environmental receptors. Data shown for a formulation containing mesotrione and s-metolachlor and several plant species. EEC or 15.3, 154, and 404 g/ha for mesotrione, metolachlor, and the formulation, respectively is based on no incorporation, high water solubility, and ground application (TerrPlant 1.2.2).

Species	Units	Mesotrione		S-Metolachlor		Sum RQ	Predicted Mixture ER50	ER50, Empirical	MDR	TU of Most Potent Active	RQ, Empirical
		Effective Conc.	RQ	Effective Conc.	RQ						
Cucumus sativa (Cucumber)	g / ha	2.43	6.28	3410	0.045	6.32	63.8	529	0.12	99.3	0.76
Lactuca sativa (Lettuce)	g / ha	0.93	16.4	4620	0.033	16.4	24.5	9.4	2.6	99.8	42.9
Avena sativa (Oat)	g / ha	210	0.073	5000	0.031	0.10	--	11500	--	--	--
Allium cepa (Onion)	g / ha	23	0.66	5000	0.031	0.69	--	950	--	--	--
Lolium perenne (Perrenial Ryegrass)	g / ha	210	0.073	1370	0.11	0.19	--	1921	--	--	--
Glycine max (Soybean)	g / ha	15.6	0.98	3970	0.039	1.02	--	280	--	--	--
Lycopersicon esculentum (Tomato)	g / ha	1.3	11.7	2750	0.056	11.8	34.2	6.4	5.3	99.5	63.1

Supplemental Information

Detailed Methods for Calculating Deviation from Mixture Model Based on Only Interlaboratory Variability

In nearly all circumstances the toxicological information used for modelling joint toxicity will have been collected independently for each of the formulation's active ingredients and for the formulation. In many cases these individual toxicity studies will have been conducted across different laboratories and/or time periods. Although the toxicological studies should have followed similar procedures based on regulatory guidelines, interlaboratory and intralaboratory variability will still occur. This variability could result in apparent deviations from toxicological models suggesting greater than or less than additive toxicity. Thus, it is important to determine the extent of deviation from the model (as measured by MDR) that may result just from the inherent variability between tests. Based on the survey of studies (Table 1 in main manuscript), coefficients of variation (CV) of 60, 100, and 140% were chosen as representative estimates for variability. To determine how these CVs would influence MDR in the case of strictly additive toxicity, we conducted a simple iterative modelling exercise.

The first step was to randomly generate a series of effective concentrations based on a distribution that had the given CV. Values were generated for each toxicity test that would be conducted in a mixture study. For example, for a binary mixture AB, a value would be generated for compound A, compound B, and mixture AB. Each value was generated assuming a mean value 1. Although all literature values of CV assumed a normal distribution, the distribution is likely log-normal. Assuming log-normal is important for modelling as effective concentrations should continue to get closer to zero, but never become negative. Thus, transformation of CV based on normal distribution to equivalent parameters in a log-normal distribution was required. The lognormal distribution is defined by the location parameter (μ) and scale parameter (σ), which can be estimated based on the normal distribution mean (m) and variability (v) by:

$$\mu = ln\left(\frac{m}{\sqrt{1+\frac{v}{m^2}}}\right) \qquad \text{Equation 1}$$

and

$$\sigma = \sqrt{ln\left(1+\frac{v}{m^2}\right)} \qquad \text{Equation 2}$$

Parameters are listed in Table 1 for the three CV values investigated. Random values were generated based on these distributions using the LOGNORM.INV function in Microsoft Excel. Probability was set as a random function [Rand()]. For each iteration, the resulting values for the active ingredients were used in Concentration Addition Model (Equation 1) and by assuming a 1:1 mix the expected EC50 for the mixture was determined.

$$ECx_{mix} = \left(\sum_{i=1}^{n} \frac{p_i}{ECx_i} \right)^{-1} \qquad \text{Equation 3.}$$

Where ECx_{mix} is the total concentration of the mixture that causes x effect; p_i indicates the proportion of component i in the mixture; and ECx_i indicates the concentration of component i that would cause x effect.

The ECx_{mix} concentration was assigned as expected concentration to mirror what is done in an actual study and the randomly generated value for the mixture was assigned as the observed EC50 and the Model Deviation Ratio (MDR) was calculated as described by the following equation:

$$MDR = \frac{Expected}{Observed} \qquad \text{Equation 4}$$

where Expected is the effective concentration of the mixture that would be predicted by the model based on the and Observed is the effective concentration for the mixture obtained from toxicity testing (Belden and Lydy, 2006).

This process was iterated 12,000 times 12,000. Based on this calculated distribution of MDR scores, the distribution of MDR values were calculated (Figure 3 and Table 2). The whole process was replicated for single, binary, and tertiary mixtures.

Table S1. Normal and estimated lognormal distribution parameters for each coefficient of variation tested. All data based on a mean of 1 for the normal distribution.

Coefficient of Variation, CV	Standard Deviation (SD)	Variability (V)	Location parameter (μ)	Scale parameter (σ)
60	0.6	0.36	-0.15374	0.55451
100	1	1	-0.34657	0.83255
140	1.4	1.96	-0.54259	1.04172

Supplemental Information

Table S2. Mixture threshold values that would require empirical testing of the formulation mixture. RQ indicates how the Risk Quotient is calculated. LOC is the level of concern. If the sum RQ for all active ingredients exceeds the mixture threshold, then empirical mixture testing is required. First four columns obtained from: https://www.epa.gov/pesticide-science-and-assessing-pesticide-risks/technical-overview-ecological-risk-assessment-risk; accessed 11/07/16.

Non-target Organism	Risk Presumption	RQ	LOC	Mixture Threshold
Birds	Acute Risk	EEC/LC_{50} or LD_{50}/ft^2 or LD_{50}/day	0.5	0.1
	Acute Restricted Use	EEC/LC_{50} or LD_{50}/ft^2 or LD_{50}/day or LD_{50} < 50 mg/kg	0.2	0.04
	Acute Endangered Species	EEC/LC_{50} or LD_{50}/ft^2 or LD_{50}/day	0.1	0.02
	Chronic Risk	EEC/NOEC	1.0	0.2
Wild Mammals	Acute High Risk	EEC/LC_{50} or LD_{50}/ft^2 or LD_{50}/day	0.5	0.1
	Acute Restricted Use	EEC/LC_{50} or LD_{50}/ft^2 or LD_{50}/day or LD_{50} < 50 mg/kg	0.2	0.04
	Acute Endangered Species	EEC/LC_{50} or LD_{50}/ft^2 or LD_{50}/day	0.1	0.02
	Chronic Risk	EEC/NOEC	1.0	0.2
Aquatic Animals	Acute High Risk	EEC/LC50 or EC50	0.5	0.1
	Acute Restricted Use	EEC/LC50 or EC50	0.1	0.02
	Acute Endangered Species	EEC/LC50 or EC50	0.05	0.01
	Chronic Risk	EEC/NOAEC	1.0	0.2
Terrestrial and Semi-Aquatic Plants	Acute High Risk	EEC/EC25	1.0	0.2
	Acute Endangered Species	EEC/EC05 or NOEC	1.0	0.2
Aquatic Plants	Acute High Risk	EEC/EC50	1.0	0.2
	Acute Endangered Species	EEC/EC05 or NOEC	1.0	0.2

Supplemental Information

Table S3. Summary of source data used to evaluate mixture toxicity

Mixture	Formulation/Compound	Species	Endpoint	Units	Concentration	Reference
1	**A13623Q**	*Daphnia magna* (Water flea)	Mobility/lethality, 48h; EC50	mg/L	0.00071	Goodband 2004
	Lambda-cyhalothrin	*Daphnia magna* (Water flea)	Mobility/lethality, 48h; EC50	mg/L	0.00036	Farrelly et al. 1984
	Thiamethoxam	*Daphnia magna* (Water flea)	Mobility/lethality, 48h; EC50	mg/L	100*	Neumann 1996
2	**A14917D**	*Daphnia magna* (Water flea)	Mobility/lethality, 48h; EC50	mg/L	100*	Liedtke 2010
	Mesotrione	*Daphnia magna* (Water flea)	Mobility/lethality, 48h; EC50	mg/L	900	Gental and Hamer 1995
	Atrazine	*Daphnia magna* (Water flea)	Mobility/lethality, 48h; EC50	mg/L	6.9	Macek 1976
3	**A15936Z**	*Daphnia magna* (Water flea)	Mobility/lethality, 48h; EC50	mg/L	19	Hoger 2009A
	Bicyclopyrone	*Daphnia magna* (Water flea)	Mobility/lethality, 48h; EC50	mg/L	100*	Batscher 2007A
	Metolachlor	*Daphnia magna* (Water flea)	Mobility/lethality, 48h; EC50	mg/L	26	Collins 1995A
4	**A16901B**	*Daphnia magna* (Water flea)	Mobility/lethality, 48h; EC50	mg/L	0.027	Kuhl and Wedra 2010A
	Cyantraniliprole	*Daphnia magna* (Water flea)	Mobility/lethality, 48h; EC50	mg/L	0.009	Samel 2004
	Thiamethoxam	*Daphnia magna* (Water flea)	Mobility/lethality, 48h; EC50	mg/L	100*	Neumann 1996
5	**A19414A**	*Daphnia magna* (Water flea)	Mobility/lethality, 48h; EC50	mg/L	74	Eser 2014
	Mesotrione	*Daphnia magna* (Water flea)	Mobility/lethality, 48h; EC50	mg/L	900	Gental and Hamer 1995
	Atrazine	*Daphnia magna* (Water flea)	Mobility/lethality, 48h; EC50	mg/L	6.9	Macek 1976
	Metolachlor	*Daphnia magna* (Water flea)	Mobility/lethality, 48h; EC50	mg/L	26	Collins 1995
6	**A14917D**	*Pseudokirchneriella subcapitata* (Green algae)	Growth Inhibition, 96h; EC50	mg/L	0.12	Liedtke 2009
	Mesotrione	*Pseudokirchneriella subcapitata* (Green algae)	Growth Inhibition, 96h; EC50	mg/L	4.2	Shillabeer et al. 1997
	Atrazine	*Pseudokirchneriella subcapitata* (Green algae)	Growth Inhibition, 96h; EC50	mg/L	0.041	Hobert 1993
7	**A15936Z**	*Pseudokirchneriella subcapitata* (Green algae)	Growth Inhibition, 96h; EC50	mg/L	0.063	Hoger 2009B
	Bicyclopyrone	*Pseudokirchneriella subcapitata* (Green algae)	Growth Inhibition, 96h; EC50	mg/L	2.2	Batscher 2007B
	Metolachlor	*Pseudokirchneriella subcapitata* (Green algae)	Growth Inhibition, 96h; EC50	mg/L	0.011	Hoberg 1995
8	**A19414A**	*Pseudokirchneriella subcapitata*	Growth Inhibition, 96h; EC50	mg/L	0.096	Falk 2014

		(Green algae)				
	Mesotrione	*Pseudokirchneriella subcapitata* (Green algae)	Growth Inhibition, 96h; EC50	mg/L	4.2	Shillabeer et al. 1997
	Atrazine	*Pseudokirchneriella subcapitata* (Green algae)	Growth Inhibition, 96h; EC50	mg/L	0.041	Hobert 1993
	Metolachlor	*Pseudokirchneriella subcapitata* (Green algae)	Growth Inhibition, 96h; EC50	mg/L	0.011	Hoberg 1995
9	**A13623Q**	*Oncorhynchus mykiss* (Trout)	Lethality, 96H; LC50	mg/L	0.011	Forbs 1992
	Lambda-cyhalothrin	*Oncorhynchus mykiss* (Trout)	Lethality, 96H; LC50	mg/L	0.00024	Hill et al. 1984
	Thiamethoxam	*Oncorhynchus mykiss* (Trout)	Lethality, 96H; LC50	mg/L	100*	Rufli 1997
10	**A15936Z**	*Oncorhynchus mykiss* (Trout)	Lethality, 96H; LC50	mg/L	7.5	Garcia et al. 2015
	Bicyclopyrone	*Oncorhynchus mykiss* (Trout)	Lethality, 96H; LC50	mg/L	100*	Batscher, R. 2007C
	Metolachlor	*Oncorhynchus mykiss* (Trout)	Lethality, 96H; LC50	mg/L	12	Collins 1995B
11	**A16901B**	*Oncorhynchus mykiss* (Trout)	Lethality, 96H; LC50	mg/L	100*	Kuhl and Wydra2010B
	Cyantraniliprole	*Oncorhynchus mykiss* (Trout)	Lethality, 96H; LC50	mg/L	13	Bouchelle 2006
	Thiamethoxam	*Oncorhynchus mykiss* (Trout)	Lethality, 96H; LC50	mg/L	100*	Rufli 1997
12	**A18219B**	*Oncorhynchus mykiss* (Trout)	Lethality, 96H; LC50	mg/L	67.5	Wiech 2012
	Mesotrione	*Oncorhynchus mykiss* (Trout)	Lethality, 96H; LC50	mg/L	110*	Kelso et al 1994
	Atrazine	*Oncorhynchus mykiss* (Trout)	Lethality, 96H; LC50	mg/L	11	Rufli 1989
13	**A19414A**	*Oncorhynchus mykiss* (Trout)	Lethality, 96H; LC50	mg/L	18	Wiech 2014
	Mesotrione	*Oncorhynchus mykiss* (Trout)	Lethality, 96H; LC50	mg/L	110*	Kelso et al 1994
	Atrazine	*Oncorhynchus mykiss* (Trout)	Lethality, 96H; LC50	mg/L	11	Rufli 1989
	Metolachlor	*Oncorhynchus mykiss* (Trout)	Lethality, 96H; LC50	mg/L	12	Collins 1995B
14	**A18484C**	*Apis mellifera* (Honey bee)	Oral Lethality, 48h; LC50	ng/bee	48	Kling 2015
	Lamba-cyhalothrin	*Apis mellifera* (Honey bee)	Oral Lethality, 48h; LC50	ng/bee	910	Gough et al. 1984
	Thiamethoxam	*Apis mellifera* (Honey bee)	Oral Lethality, 48h; LC50	ng/bee	5	Kleiner 1995
15	**A19018A**	*Apis mellifera* (Honey bee)	Oral Lethality, 48h; LC50	ng/bee	40	Kling 2012
	Cyantraniliprole	*Apis mellifera* (Honey bee)	Oral Lethality, 48h; LC50	ng/bee	110	Kling 2005
	Thiamethoxam	*Apis mellifera* (Honey bee)	Oral Lethality, 48h; LC50	ng/bee	5	Kleiner 1995
16	**A16901B**	*Colinus virginianus* (Quail)	Oral Lethality, 48h; LC50	mg/kg	2000*	Hubbard and Beavers 2
	Cyantraniliprole	*Colinus virginianus* (Quail)	Oral Lethality, 48h; LC50	mg/kg	2100*	Hubbard and Beavers 2
	Thiamethoxam	*Colinus virginianus* (Quail)	Oral Lethality, 48h; LC50	mg/kg	1600	Johnson 1996
17	**A18484C**	*Colinus virginianus* (Quail)	Oral Lethality, 48h; LC50	mg/kg	2000*	Hubbard and Frey 2015
	Lamba-cyhalothrin	*Colinus virginianus* (Quail)	Oral Lethality, 48h; LC50	mg/kg	500*	pmep.cce.cornell.edu
	Thiamethoxam	*Colinus virginianus* (Quail)	Oral Lethality, 48h; LC50	mg/kg	1600	Johnson 1996
18	**12854L**	*Cucumus sativa* (Cucumber)	Foliar Dry Mass, 21D; ER50	g / ha	38.8	Stefanut 2006

	Mesotrione	*Cucumus sativa* (Cucumber)	Foliar Dry Mass, 21D; ER50	g / ha	2.43	Porch et al. 2004
	Atrazine	*Cucumus sativa* (Cucumber)	Foliar Dry Mass, 21D; ER50	g / ha	130	Martin 2015
	S-Metolachlor	*Cucumus sativa* (Cucumber)	Foliar Dry Mass, 21D; ER50	g / ha	3410	Bramby-Gunary 2014
19	**A12909Q**	*Cucumus sativa* (Cucumber)	Foliar Dry Mass, 21D; ER50	g / ha	529	Bramby-Gunary 2016
	Mesotrione	*Cucumus sativa* (Cucumber)	Foliar Dry Mass, 21D; ER50	g / ha	2.43	Porch et al. 2004
	S-Metolachlor	*Cucumus sativa* (Cucumber)	Foliar Dry Mass, 21D; ER50	g / ha	3410	Bramby-Gunary 2014
20	**12854L**	*Lactuca sativa* (Lettuce)	Foliar Dry Mass, 21D; ER50	g / ha	41	Stefanut 2006
	Mesotrione	*Lactuca sativa* (Lettuce)	Foliar Dry Mass, 21D; ER50	g / ha	0.93	Porch et al. 2004
	Atrazine	*Lactuca sativa* (Lettuce)	Foliar Dry Mass, 21D; ER50	g / ha	65	Martin 2015
	S-Metolachlor	*Lactuca sativa* (Lettuce)	Foliar Dry Mass, 21D; ER50	g / ha	4620	Bramby-Gunary 2014
21	**A12909Q**	*Lactuca sativa* (Lettuce)	Foliar Dry Mass, 21D; ER50	g / ha	9.4*	Bramby-Gunary 2016
	Mesotrione	*Lactuca sativa* (Lettuce)	Foliar Dry Mass, 21D; ER50	g / ha	0.93	Porch et al. 2004
	S-Metolachlor	*Lactuca sativa* (Lettuce)	Foliar Dry Mass, 21D; ER50	g / ha	4620	Bramby-Gunary 2014
22	**12854L**	*Avena sativa* (Oat)	Foliar Dry Mass, 21D; ER50	g / ha	846	Stefanut 2006
	Mesotrione	*Avena sativa* (Oat)	Foliar Dry Mass, 21D; ER50	g / ha	210*	Porch et al. 2004
	Atrazine	*Avena sativa* (Oat)	Foliar Dry Mass, 21D; ER50	g / ha	280	Martin 2015
	S-Metolachlor	*Avena sativa* (Oat)	Foliar Dry Mass, 21D; ER50	g / ha	5000*	Bramby-Gunary 2014
23	**A12909Q**	*Avena sativa* (Oat)	Foliar Dry Mass, 21D; ER50	g / ha	11500	Bramby-Gunary 2016
	Mesotrione	*Avena sativa* (Oat)	Foliar Dry Mass, 21D; ER50	g / ha	210*	Porch et al. 2004
	S-Metolachlor	*Avena sativa* (Oat)	Foliar Dry Mass, 21D; ER50	g / ha	5000*	Bramby-Gunary 2014
24	**12854L**	*Allium cepa* (Onion)	Foliar Dry Mass, 21D; ER50	g / ha	54*	Stefanut 2006
	Mesotrione	*Allium cepa* (Onion)	Foliar Dry Mass, 21D; ER50	g / ha	23	Porch et al. 2004
	Atrazine	*Allium cepa* (Onion)	Foliar Dry Mass, 21D; ER50	g / ha	190	Martin 2015
	S-Metolachlor	*Allium cepa* (Onion)	Foliar Dry Mass, 21D; ER50	g / ha	5000*	Bramby-Gunary 2014
25	**A12909Q**	*Allium cepa* (Onion)	Foliar Dry Mass, 21D; ER50	g / ha	950	Bramby-Gunary 2016
	Mesotrione	*Allium cepa* (Onion)	Foliar Dry Mass, 21D; ER50	g / ha	23	Porch et al. 2004
	S-Metolachlor	*Allium cepa* (Onion)	Foliar Dry Mass, 21D; ER50	g / ha	5000*	Bramby-Gunary 2014
26	**12854L**	*Lolium perenne* (Perennial Ryegrass)	Foliar Dry Mass, 21D; ER50	g / ha	353	Stefanut 2006
	Mesotrione	*Lolium perenne* (Perennial Ryegrass)	Foliar Dry Mass, 21D; ER50	g / ha	210*	Porch et al. 2004
	Atrazine	*Lolium perenne* (Perennial Ryegrass)	Foliar Dry Mass, 21D; ER50	g / ha	1200	Martin 2015
	S-Metolachlor	*Lolium perenne* (Perennial Ryegrass)	Foliar Dry Mass, 21D; ER50	g / ha	1370	Bramby-Gunary 2014
27	**A12909Q**	*Lolium perenne* (Perennial Ryegrass)	Foliar Dry Mass, 21D; ER50	g / ha	1921	Bramby-Gunary 2016
	Mesotrione	*Lolium perenne* (Perennial Ryegrass)	Foliar Dry Mass, 21D; ER50	g / ha	210*	Porch et al. 2004
	S-Metolachlor	*Lolium perenne* (Perennial Ryegrass)	Foliar Dry Mass, 21D; ER50	g / ha	1370	Bramby-Gunary 2014
28	**12854L**	*Glycine max* (Soybean)	Foliar Dry Mass, 21D; ER50	g / ha	54*	Stefanut 2006

	Mesotrione	*Glycine max* (Soybean)	Foliar Dry Mass, 21D; ER50	g / ha	15.6	Porch et al. 2004
	Atrazine	*Glycine max* (Soybean)	Foliar Dry Mass, 21D; ER50	g / ha	66	Martin 2015
	S-Metolachlor	*Glycine max* (Soybean)	Foliar Dry Mass, 21D; ER50	g / ha	3970	Bramby-Gunary 2014
29	**A12909Q**	*Glycine max* (Soybean)	Foliar Dry Mass, 21D; ER50	g / ha	280	Bramby-Gunary 2016
	Mesotrione	*Glycine max* (Soybean)	Foliar Dry Mass, 21D; ER50	g / ha	15.6	Porch et al. 2004
	S-Metolachlor	*Glycine max* (Soybean)	Foliar Dry Mass, 21D; ER50	g / ha	3970	Bramby-Gunary 2014
30	**12854L**	*Lycopersicon esculentum* (Tomato)	Foliar Dry Mass, 21D; ER50	g / ha	23	Stefanut 2006
	Mesotrione	*Lycopersicon esculentum* (Tomato)	Foliar Dry Mass, 21D; ER50	g / ha	1.3	Porch et al. 2004
	Atrazine	*Lycopersicon esculentum* (Tomato)	Foliar Dry Mass, 21D; ER50	g / ha	93	Martin 2015
	S-Metolachlor	*Lycopersicon esculentum* (Tomato)	Foliar Dry Mass, 21D; ER50	g / ha	2750	Bramby-Gunary 2014
31	**A12909Q**	*Lycopersicon esculentum* (Tomato)	Foliar Dry Mass, 21D; ER50	g / ha	6.4	Bramby-Gunary 2016
	Mesotrione	*Lycopersicon esculentum* (Tomato)	Foliar Dry Mass, 21D; ER50	g / ha	1.3	Porch et al. 2004
	S-Metolachlor	*Lycopersicon esculentum* (Tomato)	Foliar Dry Mass, 21D; ER50	g / ha	2750	Bramby-Gunary 2014

*Non-definitive (unbounded) values - studies that did not result in a statistically significant effect within the range of exposures tested

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